Redistribution of Amino Acids and Amides during Seedling Development in *Acacia iteaphylla* F. Muell. (Fabaceae: Mimosoideae)

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The distribution of free amino compounds in the cotyledons and the organs derived from the embryonic axis has been studied in developing seedlings of *Acacia iteaphylla*. The results indicate that asparagine and pipecolic acid are the major forms of nitrogen translocated initially from the cotyledons to the hypocotyl and primary root. Selective transport of several 'non-protein' amino acids was indicated. *S*-carboxyethylcysteine became prominent in the hypocotyl after the pipecolic acid content of the hypocotyl had declined; it appeared also in the primary leaf, but was not strongly represented in the roots. *S*-carboxyisopropylcysteine appeared late in the hypocotyl, and was not detected in any other part of the axis. Albizziine was not transported out of the cotyledons, but like arginine was metabolized *in situ*.

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INTRODUCTION

The cotyledons of *Acacia* seedlings undergo a transition from storage organs to photosynthetic organs (Ashcroft and Murray, 1979; Murray, 1981; Smith, 1981). During this transition, reserve materials appear to be mobilized in two stages. The first encompasses the initial growth of the radicle and hypocotyl, resulting in the elevation of the cotyledons. In *Acacia iteaphylla* F. Muell., the dry matter content of the cotyledons is depleted by about 70% during this stage, but only a slight net decline in the protein content of the cotyledons is observed (Ashcroft and Murray, 1979). In the second phase, more rapid net breakdown of protein in the cotyledons is associated with the synthesis of chloroplasts, as the cotyledons and then the primary leaf become effective photosynthetic organs.

It has long been known that *Acacia* seeds contain a high proportion of total seed nitrogen as free amino acids (Petrie, 1908, 1911). Many 'non-protein' amino acids have now been identified (Seneviratne and Fowden, 1968), and their distribution among species has indicated that there are four main sub-groups of *Acacia* throughout the world (Evans *et al.*, 1977; Murray, 1986b). In view of the early slow rate of net decline in the protein content of cotyledons, Ashcroft and Murray (1979) suggested that non-protein amino acids might represent the earliest mobilized form of nitrogenous reserve. Evidence has now been obtained that some, but not all, of the non-protein amino acids are transmitted to the axis, together with newly-synthesized asparagine.

MATERIALS AND METHODS

The seeds of *Acacia iteaphylla* were from the same batch studied previously (Ashcroft and Murray, 1979). To permit uniform imbibition, the seedcoats were cut with a razor blade at the end furthest from the embryonic axis. Except for seeds imbibed for 24h, which were placed in Petri dishes between moist Whatman No. 1 filter paper at 23°C (Krishna and Murray, 1988), seeds were placed in trays of sandy soil in a glass house,

with day temperatures around 30°C. At intervals up to 14 days, groups of 10 to 20 uniform seedlings were removed, dismembered and analysed.

Extracts from cotyledons, radicles and hypocotyls were prepared using a chilled mortar and pestle, acid-washed sand, and medium consisting of 25mM K^+ phosphate (pH 7.5), 0.5mM 2-mercaptoethanol and 0.03% (w/v) Triton X-100 (Murray and Kennedy, 1980; Murray, 1983). The ratio of medium to tissue was 5:1 (mL per g fresh weight). The homogenates were centrifuged for 4min at 9,000*g* in a Microfuge and the supernatants removed. These were treated with ethanol (4:1, v/v) and insoluble material removed by centrifugation (Murray and McGee, 1986). Smaller samples (whole axes; balance of shoot) were extracted directly with ethanol (4:1, v/v). Aliquots of the ethanol-soluble fractions were assayed for amino nitrogen content by the procedure of Schnarrenberger *et al.* (1972), using L-serine as a reference standard (Murray, 1983).

Ethanol-soluble fractions were dried in a rotary evaporator, then redissolved in 0.5 to 1.0mL of 70% (v/v) ethanol, and applied to the origins of Whatman No. 1 papers prepared for 2-dimensional descending chromatography (Murray, 1983). Extracts available only in small quantities were applied without prior concentration. The solvents employed were 80% (w/v) phenol-water plus ammonia (200:1, v/v) in the first dimension, then either *n*-butanol: acetic acid: water 12:3:5 (v/v) or *n*-butanol: propionic acid: water 6:3:4 (v/v) in the second (Murray *et al.*, 1971; Murray, 1983). At least two chromatograms were run for each extract. Amino compounds were detected by their reaction with ninhydrin, and identified by comparison of their positions with those of authentic compounds. Information on the chromatographic behaviour of non-protein amino acids was kindly provided by Dr C. S. Evans and Prof. E. A. Bell. Authentic albizziine was purchased from Aldrich; other amino acids and amides were from Sigma Chemical Co.

Results

The rate of seedling growth was faster than in the previous study because of higher temperatures and longer day-length. The 4-day, 8-day and 14-day stages chosen for analysis closely resembled the 8-day, 15-day and >21-day stages described previously (Ashcroft and Murray, 1979; Murray, 1981). At the 1-day stage, the amino nitrogen content of the cotyledons effectively represents that of the whole embryo (Fig. 1), as the axis was too small for accurate analysis (less than 1mg fresh weight). The amount of free amino nitrogen in the whole seedling progressively increased, doubling by 14 days (Fig. 1). Within the cotyledons, the amino nitrogen content did not alter substantially until between 8 and 14 days, when it declined. Within the axis, the hypocotyl gained a much higher proportion of the amino nitrogen exported by the cotyledons than did the radicle.

The amino acids and amides present in *Acacia* seedling tissues were identified as shown in Fig. 2. Changes in their relative abundance in the cotyledons are shown in Table 1. Many of the major nitrogenous solutes stored in the cotyledons initially are still among the most prominent forms present after 14 days, when the total free amino nitrogen content of the cotyledons had declined by 40% (Fig. 1). The content of asparagine increased, while the contents of albizziine, glycine, arginine, pipecolic acid, and finally glutamate and alanine, declined (Table 1).

The distribution of amino compounds in the roots and hypocotyls of seedlings aged 8 and 14 days is shown in Table 2. Pipecolic acid and asparagine were the most abundant forms of free amino nitrogen in both the roots and the hypocotyl of the 8-day-old seedlings. At this stage, *S*-carboxyethylcysteine was prominent in the hypocotyl, but was not detectable in the roots. At the 14-day stage, both *S*-carboxyethylcysteine and *S*-carboxyisopropylcysteine became prominent in the hypocotyl. The content of asparagine was

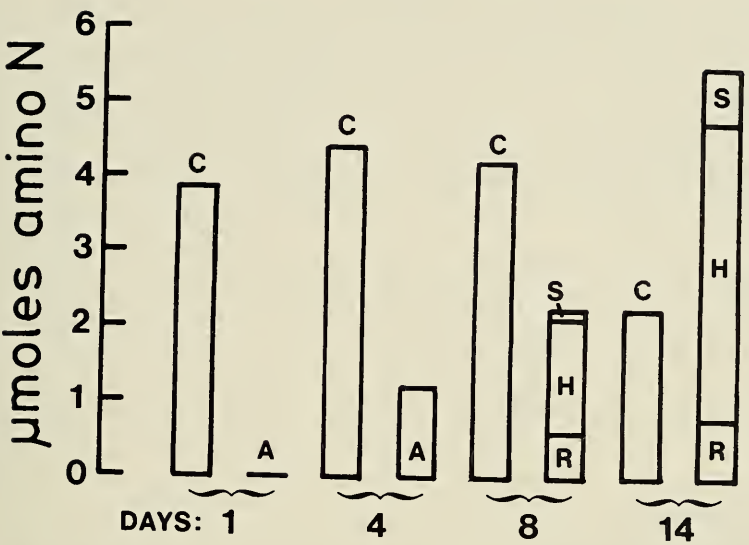

Fig. 1. Changes in the distribution of free amino nitrogen in the developing seedling of *Acacia iteaphylla*. C = cotyledon pair; A = whole embryonic axis; R = radicle or root system; H = hypocotyl; S = shoot system above the cotyledons.

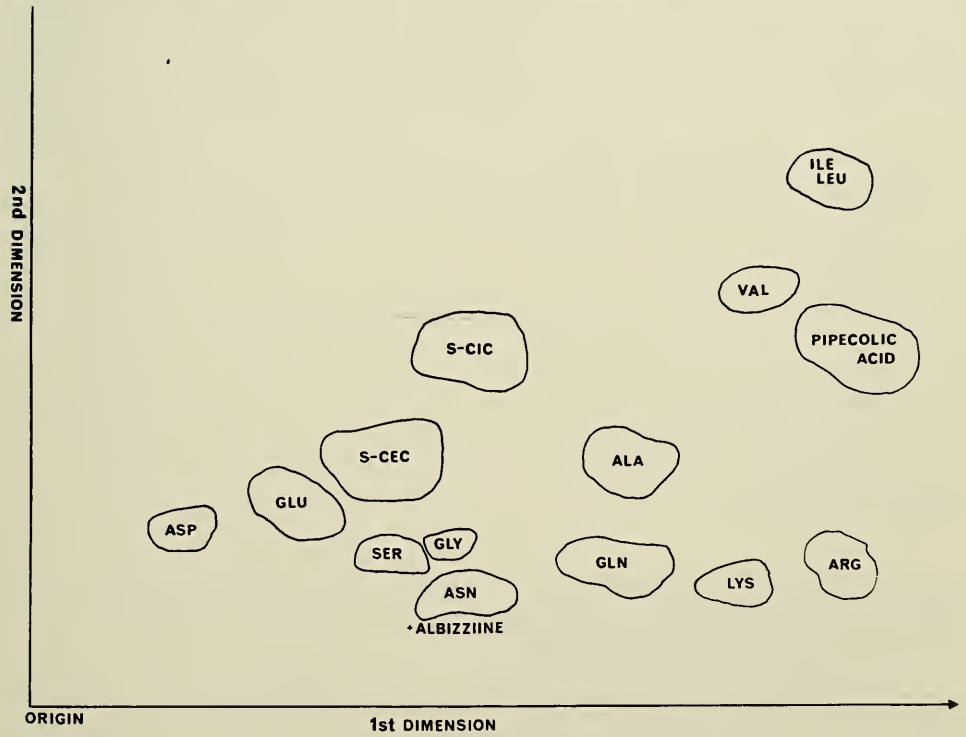

Fig. 2. The positions and identities of amino compounds separated from cotyledons of *Acacia iteaphylla* by 2-dimensional descending paper chromatography. Solvent 1, phenol-water-ammonia; solvent 2, *n*-butanol-acetic acid-water (see Materials and Methods). Abbreviations are standard, plus S-CEC = *S*-carboxyethylcysteine; S-CIC = *S*-carboxyisopropylcysteine.

TABLE 1

Changes in the Distribution of Free Amino Acids and Amides in the Cotyledons of Acacia iteaphylla *Seedlings*

Compound	Age of seedling (days) 3	4	8	14
S-carboxyethylcysteine	+ + + +	+ + + +	+ + + +	+ + +
S-carboxyisopropylcysteine	+ + +	+ + + +	+ + + +	+ + +
Pipecolic acid	+ + +	+ + + +	+ + +	+ +
Albizziine + asparagine	+ + [1]	+ +	+ + [2]	+ + + [2]
Glutamine	+ +	+ +	+ + +	+ +
Aspartate	tr	+ +	tr	+
Glutamate	+ + +	+ + +	+ + +	+
Serine	+ +	+ +	+ +	+ +
Glycine	+ +	+	+	+
Alanine	+ +	+ + +	+ +	tr
Valine	+	tr	tr	tr
Leucine + Isoleucine	+	tr	tr	tr
Lysine	tr	tr	tr	tr
Arginine	+ +	+	tr	n.d.

tr, trace; n.d., not detected.
[1] albizziine predominant;
[2] asparagine predominant — see text.

TABLE 2

Changes in the Distribution of Free Amino Acids and Amides in the Roots and Hypocotyl of Acacia iteaphylla *Seedlings*

Compound	Roots 8-day	14 day	Hypocotyl 8-day	14-day
S-carboxyethylcysteine	n.d.	+	+ +	+ + +
S-carboxyisopropylcysteine	n.d.	n.d.	n.d.	+ +
Pipecolic acid	+ + +	+ +	+ + + +	+
Asparagine	+ + +	+ +	+ + + +	+ + +
Glutamine	+ +	n.d.	+ +	n.d.
Aspartate	+	+	+ +	+
Glutamate	+	n.d.	+ +	tr
Serine	+	+ +	+ +	+
Glycine	tr	+ +	+	n.d.
Alanine	+ +	+ +	+ + +	tr
Valine	+ +	n.d.	+	n.d.
Leucine + Isoleucine	tr	n.d.	+	n.d.
Threonine	n.d.	n.d.	tr	n.d.

tr, trace; n.d., not detected.

maintained, but the contents of pipecolic acid and alanine declined sharply (Table 2). In the roots of the 14-day-old seedlings, some *S*-carboxyethylcysteine was present, but *S*-carboxyisopropylcysteine was not detected.

In the balance of the shoots from these seedlings (mainly the primary leaf at 14 days), serine and *S*-carboxyethylcysteine were most prominent. Pipecolic acid was also detected, along with aspartate, glycine, alanine, glutamate and glutamine, but asparagine, albizziine and *S*-carboxyisopropylcysteine were not detected.

Discussion

The net increase in free amino nitrogen in the seedling between 1 and 8 days was 2.55μmol (Fig. 1), which is within the maximum amount that could be met from limited breakdown of proteins in the cotyledons (Ashcroft and Murray, 1979). Our observations are consistent with the transport of selected forms of free amino nitrogen from the cotyledons to the hypocotyl, the root, and later the shoot, with the content of free amino nitrogen maintained close to 2μmol per cotyledon by proteolysis until after 8 days (Fig. 1). It must be noted that it is not possible for any uptake of exogenous nitrogen sources to have occurred through the roots; the axis-derived organs are gaining nitrogen entirely at the expense of the cotyledons.

Since the only way that the cotyledons can export nitrogenous solutes is through the phloem (Guardiola and Sutcliffe, 1972; Murray, 1984), an important question raised is whether the different organs of the axis are selective in the nitrogenous solutes they import, and if so, whether restrictions apply to phloem loading or unloading of individual compounds. In seedlings of jack bean (*Canavalia ensiformis* [L.] DC), Rosenthal and Rhodes (1984) have demonstrated that the non-protein amino acid L-canavanine is transported only to the above-ground parts of the shoot — none is translocated to the radicle.

Of all the non-protein amino acids potentially available from the cotyledons, only pipecolic acid appeared to be imported by very young roots of *Acacia iteaphylla* seedlings. Pipecolic acid and asparagine were abundant early in both the roots and the hypocotyl; quantitatively, they are the most important forms of nitrogen initially transferred from the cotyledons to the axis (Table 2). Outside the cotyledons, *S*-carboxyisopropylcysteine was detected only in the hypocotyl, relatively late in development (Table 2), whereas transport of the other derivative of cysteine was less restricted. *S*-carboxyethylcysteine became one of the more prominent nitrogenous solutes of the hypocotyl, and appeared also in the roots (late) and the primary leaf. The serine present in the primary leaf has probably accumulated from at least two biosynthetic pathways operating in the leaf itself (Murray, 1986a).

Arginine was not transported from the cotyledons (Table 2), but converted to other nitrogenous solutes *in situ* (Table 1). Urease was detected immunochemically in cotyledon extracts (for methods see Murray and Knox, 1977), which is consistent with this interpretation.

Finally, what is the role of albizziine (L-α-amino-β-ureidopropionic acid), a compound whose synthesis in *Acacia* cotyledons is known to be confined to seed development (Seneviratne and Fowden, 1968)? In our chromatographic systems, albizziine and asparagine could not be resolved (Fig. 2). This is in agreement with data on the mobility of albizziine reported by Cooper and Meister (1973). However, the colour of the reaction product with ninhydrin produced by authentic albizziine after paper chromatography was always mauve, whereas that produced by asparagine was always brown. On chromatograms where authentic albizziine and asparagine were loaded together, the final colour reflected the abundance of each compound; a brown colour consistently indicated the predominance of asparagine.

Initially albizziine is the prominent component of the combined albizziine-asparagine area from cotyledon extracts, but with increasing time following germination, asparagine predominates. When detected on chromatograms of *Acacia* root and hypocotyl extracts, the joint region was always brown, and if the unstained area was eluted from replicate chromatograms and hydrolysed with HCl, a ninhydrin positive product with the same mobility as aspartate was recovered. We have therefore concluded that asparagine is a major nitrogenous solute translocated to the roots and

hypocotyl (Table 2). This is in agreement with a transport function for newly synthesized asparagine determined for cotyledons of light-grown pea (Melcher, 1983) and peanut seedlings (Peoples *et al.*, 1986).

In *Acacia* cotyledons albizziine, like arginine, is converted to translocated forms of nitrogen, predominantly asparagine. It remains to be determined exactly how albizziine is metabolized, and whether, while present in cotyledons, it has any deterrent function against larvae of Australian insects.

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